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Translational Research

Principal Investigator: Bakay, Roy A
Grant Number: 1R01NS046612-01A1
Title: Stem Cells in CNS Transplantation

Abstract: Stem cells offer tremendous promise for the future of transplantation. We propose examining embryonic stem cells (ESC) in monkey allografts. We will compare dopaminergic enriched ESC to fetal mesencephalic (FM) neurons in their ability to survive, innervate, and restore lost function in the best animal model of PD, the MPTP treated monkey. The primate is essential for this study to test the hypothesis that replacement strategy must completely reinnervate the very large volume of the monkey striatum. Recently clinical trials have indicated that dopaminergic (DAergic) replacement with FM neurons can cause severe debilitating dyskinesia. It is then imperative to have a clear understanding of how a DAergic enriched ESC replacement strategy affects I-dopa-induced dyskinesia (LID). In this regard, we will also compare the effects of FM transplants and DAergic enriched ESC upon the dyskinesia profile of MPTP monkeys. The potential to induce or diminish dyskinesia will be tested with the best model of dyskinesia (primate LID model). The key problem of parkinsonian transplantation with fetal or stem cells grafts is the incomplete reinnervation of host striatum. Like the FM transplant patients, focal areas of relative hyperdopaminergic activity should render these monkeys highly susceptible to LIDs. Thus to optimize reinnervation and functional recovery while minimizing the potential for dyskinesia, we will also treat DAergic enriched ESC with glial cell line-derived neurotrophic factor (GDNF) delivered via a lentiviral vector. The lenti-viral vector is critical to this hypothesis because of the proven ability to transfect the entire striatum and act not as a point source but as a volume source to stimulate reinnervation. Intraparenchymal GDNF released diffusely throughout the entire striatum should act as a developmental cue for these immature cells to extend DAergic processes throughout the striatum as well as provide neuronal rescue for dopaminergic neurons in the pars compacta of the substantia nigra. Sufficient subjects and multiple controls are included to insure proper interpretation of the data. The present series of experiments serves to provide the essential preclinical data needed to help determine the utility of nonhuman dopaminergic enriched stem cells. -

Principal Investigator: BULTE, JEFF W
Grant Number: 5R01NS045062-02
Title: MR Tracking of Magnetically Labeled Stem Cells in CNS

Abstract: Unavailable

Principal Investigator: DURING, MATTHEW J
Grant Number: 1R01NS044576-01
Title: Somatic Cell Gene Transfer/Neurological & Clin Applics

Abstract: Gene transfer in the mammalian nervous system has been the primary research focus of our laboratory for the past decade. We are excited that this RFA has come at a time when the field is flourishing, yet clinical translation remains daunting, and much work needs to be done for ultimate success in the clinic. In this grant application we propose to focus on some of the more pressing needs using rat models of Parkinson Disease. Our first aim is to further develop more efficient and readily packaged and purified AAV vectors for clinical translation. Here, we will characterize and compare pseudotyped and chimeric AAV vectors and in addition develop novel reagents, including helper plasmids and protocols which can be used by the entire gene therapy community to more efficiently generate these vectors. Our preliminary data suggests that these new chimeric and pseudotyped vectors represent a significant advance above our current generation rAAV-2 vectors. Secondly, we will develop optimal expression cassettes with a focus on promoter; post regulatory sequences as well as elements like the human beta-interferon scaffold attachment region (SAR) to boost expression. Thirdly, we will further develop a regulatable system. We present in our preliminary data our latest generation bi-directional tet cassette with tandem minimal insulator sequences flanking the vector genome. Here we propose to use this vector as the starting point to develop a novel cassette with the use of KRAB-AB domain from kid-1 as a suppressor. Our fourth aim is the use of rAAV to over express PAEL receptor in the adult rat substantial nigra with characterization of the phenotype as a potential genetic model of Parkinson Disease. Finally, we propose the use of a picospritzer and in vivo single unit recording to develop methods for focal and electrophysiological mapped neuronal gene delivery. We will target the substantia nigra pars compacta, using AAV expressing wildtype parkin, as a potential therapy for parkin mutation associated, autosomal recessive Parkinson Disease (AR-PD) as modeled by the PAEL receptor over expressing rats as developed in specific aim 4.-

Principal Investigator: FEDEROFF, HOWARD J.
Grant Number: 5R01NS036420-08
Title: Improved HSV Vectors: Gene Transfer into Nervous System

Abstract: Gene transfer methods have created the opportunity for developing gene therapy for human neurological diseases such as Parkinson's Disease (PD). Since PD represents a group of clinically similar syndromes each triggered by a different mechanism we hypothesize the existence of a shared downstream pathophysiologic pathway. Our goal is to develop therapy for PD directed at a shared common node in the pathway. The elaboration of such neuroprotective gene therapy is contingent on the development of safe and efficacious gene transfer vectors that can express a therapeutic gene for a prolonged period in specific neuronal populations. Of the currently available vehicles for direct gene therapy only plasmid based herpes simplex virus (HSV) "amplicon" vectors have been demonstrated to both accommodate a large (9 kb) tyrosine hydroxylase (TH) promoter fragment and to provide highly selective gene expression in dopamine (DA) neurons in the substantia nigra. However, HSV amplicon vectors exhibit transgene silencing that is an impediment to one-time dosing for a chronic disease such as PD. Our data indicate that transgene silencing results from heterochromatin formation. One of the goals of this project is to subvert transgene silencing by altering the propensity of vector to form heterochromatin. In Specific Aim 1 we examine multiple different approaches to stimulate euchromatin formation, that chromatin state posited to support long term gene expression. A second issue pertinent to the development of PD gene therapy is to direct different therapeutic genes to each compartment of the diseased nigrostriatal pathway: dopamine neurons and target striatum. In Specific Aim 2 we will develop separate vectors which will afford direct expression of different gene products to each anatomical compartment. A third issue for successful PD gene therapy is evaluation in appropriate animal models of the disease. Specific Aim 3 will employ two animal models: Our novel a-synuclein mice which develop progressive nigrostriatal dysfunction, reduction of substantia nigra TH and hypokinetic activity; and our modified chronic MPTP model which produces striatal denervation, dopaminergic cell loss and a neurobehavorial syndrome. The proposed studies will yield optimized HSV vectors, provide a detailed understanding of their characteristics, and evaluate their effectiveness in mechanistically different models of PD. -

Principal Investigator: FEDEROFF, HOWARD J.
Grant Number: 5U54NS045309-03
Title: Parkinson's Disease Gene Therapy Study Group

Abstract: Parkinson's disease (PD) affects about 1 million people in North America. Medications, such as levodopa, and some surgical approaches are available for PD, but offer only symptomatic therapy. New information contribute to current optimism that gene therapy might correct the molecular disturbances of PD, alleviate the symptoms of the illness and/or in retarding disease progression. Setbacks in gene therapy for other diseases underscore the importance of a purposely deliberate and careful approach that demands substantial assurances of safety and potential efficacy in advance of human testing. It is this philosophy of conservatism that will characterize the activities of our group. A coordinated stepwise progression from basic research through exhaustive preclinical evaluation prior to clinical testing is required. A multicenter, multidisciplinary collaborative group (The PD Gene Therapy Study Group [PDGTSG]) has formed and seeks support for those activities that will lead to a large-scale clinical trial of gene therapy for patients with PD. The PDGTSG consists of three different components: Cores, Principal Projects, and Pipeline Projects. Core A. Administrative Core (PI: Dr. Federoff): Houses a Steering Committee, and Vector (Chair: Dr. Lowenstein), Human Subjects/Clinical Assessment (Chair: Dr. Kurlan), Bioethics (Chair: Ms. Greenlaw), Intellectual Property (Chair: Ms. Hunter) and Biostatistics Modules (Chair: Dr. Oakes). Provides for the coordination of budgeting, committee scheduling, reports, progress preparation, and interface with NINDS staff, the clinical, scientific and lay community. Core B. Biological Measurement Core (PI: Dr. Federoff: Functions in the application shared quantitative measurements. Houses the database and the bank of vector constructs used in all studies. Project I. "Enzymatic Gene Transfer in MPTP Monkeys" (PIs: Bankiewicz and Kordower) Will comprehensively evaluate two vector platforms (rHIV and rAAV), each transducing the identical AADC gene cassette in the standardized non-human primate model. Project II. "Trophic Gene Transfer in MPTP Monkeys" (PIs: Bankiewicz and Kordower) Will comprehensively evaluate two vector platforms (rHIV and rAAV), each transducing the identical regulated GDNF gene cassette in the standardized non-human primate model. PIPELINE PROJECTS 0PPs) Focus 1: 1reproved regulation of gene expression PP I. "Tet-Regulated Vectors for Parkinson's Disease" (PI: Bohn). PP II. "Engineering RNA Switches that Respond to Dopamine and its Analogs" (PI: Breaker). Focus 2: Development of new vector platforms for application in PD disease models. PP III. "High Capacity Gutless Adenovirus" (PI: Lowenstein). PP IV. "Development of Integrating HSV

Principal Investigator: IACOVITTI, LORRAINE M
Grant Number: 3R21NS043705-02S1
Title: Neural Stem Cells Grafts in Primate Models of Parkinsons

Abstract: Unavailable

Principal Investigator: ISACSON, OLE
Grant Number: 3P50NS039793-05S1
Title: NOVEL THERAPEUTIC APPROACHES FOR PARKINSON'S DISEASE

Abstract: Unavailable

Principal Investigator: KANG, UN Jung
Grant Number: 5R01NS032080-11
Title: Dopamine regulation in parkinsonian rat by gene therapy

Abstract: L-3,4-dihydroxyphenylalanine (L-DOPA) is the mainstay of therapy for Parkinson's disease (PD). Chronic L-DOPA therapy is limited, however, by the development of motor response complications, such as progressively shorter duration of improvement in akinesia (wearing-off) and the appearance of L-DOPA-induced abnormal involuntary movements. Innovative methods of sustained and localized central nervous system (CNS) dopamine delivery may further optimize L-DOPA therapy. Such methods are being explored clinically by CNS transplantation studies with fetal dopaminergic neurons and experimentally by neuronal stem cell implants and gene therapy. Our studies during the past funding cycles have defined optimal sets of genes necessary for dopamine replacement using ex vivo gene therapy using genetically modified fibroblasts. We also developed rat behavioral models that are relevant to the akinesia of PD patients. Using akinesia behaviors, we have noted that lesion severity has a major influence on the shortening of the response duration with minor contribution by the chronic intermittent L-DOPA therapy. Therefore, studies proposed in this continuing renewal application will determine the optimal parameters of gene therapy to improve akinesia and minimize and prevent motor response complications. We will use adeno-associated virus vectors to deliver tyrosine hydroxylase and guanosine triphosphate (GTP) cyclohydrolase 1 genes. The optimal combination of anatomical targets for gene therapy to improve akinesia will be defined by examining the effects of gene therapy delivered to basal ganglia structures, such as subthalamic nucleus, substantia nigra par reticulata, that receive dopaminergic inputs, in addition to the striatum. The optimal timing to initiate dopamine replacement gene therapy to forestall development of motor response complications will also be examined. These results will have significant implications beyond dopamine replacement gene therapy proposed here and guide other therapies such as fetal dopaminergic cell transplantation, neurotrophic factor therapy, stem cell therapy, and other CNS targeted delivery systems. -

Principal Investigator: KIM, KWANG S
Grant Number: 5R21NS044439-02
Title: DA-specific gene discovery and promoter engineering

Abstract: Gene therapy techniques need substantial development to provide therapeutic possibilities for treating neurological disorders such as Parkinson's disease (PD). Based on molecular control mechanisms of noradrenergic neuron-specific gene regulation, we recently devised a gene delivery system that can efficiently target transgene expression to noradrenergic neurons in a cell-specific manner. Our long-term goal is to establish gene therapy system(s) that will drive efficient transgene expression in a dopamine (DA) neuron-specific fashion based on discovery and characterization of DA-specific genes. Toward this end, we propose to identify and isolate genes that are selectively expressed in the DA mid-brain area by analyzing gene expression profiles using the most comprehensive cDNA microarrays such as the augmented NIA 16K chip and augmented RIKEN 16 K chip. Because these chips do not cover the whole genome yet, we will also identify novel DA-specific genes by the PCR-based subtractive hybridization techniques. Expression patterns of putative DA-specific genes will be tested by semi-quantitative RT-PCR using independently isolated mRNAs, and will be confirmed by in situ hybridization. Among the isolated DA-specific genes, we will first focus on putative DNA-binding transcription factors. The consensus binding sites for these putative transcription factors will be defined and their potential promoter function will be tested by cotransfection assays using cell line systems. On the basis of the mechanism of action of the novel DA-specific transcription factor(s), synthetic promoters will be developed and optimized. The optimized synthetic promoter will be subcloned in front of the reporter lacZ gene in the context of the self-inactivated lenti viral vectors. Cell type-specific expression of the reporter gene will be examined using both in vitro mesencephalic primary neuronal cultures as well as in different rat brain areas following stereotactic injection. At the later stage of this proposal, we will plan to use our developed promoter system(s) to deliver therapeutic genes (e.g., GDNF and Bcl 2) to the DA neurons and will test whether they can efficiently ameliorate behavioral symptoms in animal models of PD. The proposed research will identify and isolate genes that are selectively expressed in the mid-brain DA area on a genome-wide scale and will characterize their transcriptional regulation. Based on these mechanisms, we will devise novel and innovative DA-specific promoter systems and test them using in vitro and in vivo systems. In combination with safe viral vectors, our developed gene delivery systems can be translated clinically into gene therapy approaches for PD and other neurological disorders, in which DA

Principal Investigator: KORDOWER, JEFFREY H
Grant Number: 5R01NS043290-03
Title: DYSKINESIAS IN LENTI-GDNF TREATED PARKINSONIAN MONKEYS

Abstract: Fetal nigral grafts can cause "runaway" dyskinesias in patients with Parkinson's disease (PD;Freed et al., 2001). These dyskinesias are severe, debilitating and strongly indicate that 1) novel dopaminergic surgical therapeutic strategy planned for clinical trials need to be tested preclinically for their effects upon dyskinesias and 2) the mechanisms underlying these dyskinesias need to be elucidated. We have recently demonstrated that lentiviral gene delivery of glial cell-derived neurotrophic factor (GDNF) potently prevents motor dysfunction and prevents nigrostriatal degeneration in nonhuman primate models of PD (Kordower et al., 2000). Prior to initiating clinical trials with lenti-GDNF, it effects upon dyskinesias need to be evaluated in parkinsonian monkeys. Freed, Fahn and coworkers (2001) have hypothesized that grafted-mediated dyskinesias result from graft overgrowth. However, their own PET and post-mortem data, as well as the data from others (Kordower et al., 1995, Lee et al 1999), do not support this view. We propose an alternative hypothesis that these dyskinesias result from local "hot spots" of hyperdopaminergic function interacting with the levodopa primed brain. We plan to test this hypothesis by comparing gene therapies that induce either a) widespread or b) local hyperdopaminergic function upon dopa-induced dyskinesias and the role of dopa priming. This application will have three Specific Aims. Specific Aim 1 will test the hypothesis that lenti-GDNF treatment to non-levodopa primed MPTP-treated monkeys will prevent, or diminish the intensity of dyskinesias when they are later treated with levodopa. Specific Aim 2 will test the hypothesis that lenti-GDNF will diminish the dyskinesia profile in dyskinesic MPTP-treated monkeys previously primed with levodopa. Specific Aim 3 will test the hypothesis that "hot- spot" hyperdopaminergic function, but not homogenous hyperdopaminergic innervation, will enhance the dyskinesia profile of parkinsonian monkeys and that elimination of GDNF will reverse the functional and dyskinesic effects established previously by this trophic factor. The study of dyskinesias has become a compelling area of PD research. Exciting therapeutic strategies such as gene therapy need to be evaluated for their effects on dyskinesias so that they are both safe and effective. This application will determine whether potent dopaminergic gene therapies influence dyskinesias in the best animal model of PD.-

Principal Investigator: LAU, YUEN-SUM
Grant Number: 5R01NS047920-02
Title: Impact of Exercise on Parkinson's Disease Therapy

Abstract: Parkinson's disease (PD) is a slow, progressive, debilitative, neurodegenerative disease, which has no cure. The current pharmacological therapies only temporarily mask symptoms, but do not protect neurons from further degeneration. Furthermore, chemotherapeutic agents often cause severe adverse effects and reduce the effectiveness of treatment. Numerous clinical reports have suggested that endurance exercise can slow down disease progression, and add years of independent and quality life to PD patients, or even improve the delivery and efficacy of L-DOPA treatment. Exercise therapy, or in conjunction with drug therapy at early onset of disease state, have been highly advocated by recent clinical trials. The potential health benefit and neurological mechanisms of action for exercise on PD rehabilitation have not been rigorously tested in the laboratory animal models. This research is designed to elucidate the impact of endurance exercise training on nigrostriatal dopamine (DA) neuron plasticity using a slow, progressive, and neurodegenerative mouse model of PD developed and characterized by our laboratory. This model is established based on a regimen of chronic 1-Methyl-4-phenyl - 1,2,3,6-tetrahydropyridine (MPTP) injections co-administered with probenecid, a drug that inhibits the peripheral and neuronal clearance of MPTP and potentiates the neurotoxicity of MPTP. In this model, we observed a marked decrease of nigrostriatal DA function within one week after treatment and remained low for 6 months. The animal also shows a gradual loss of substantia nigra (SN) neurons, decline of motor activity, and an accumulation of c-synuclein-immunoreactive inclusions in the SN. We further present in the application our preliminary findings supporting the feasibility and potential neuromodulatory role of endurance exercise on enhancing nigrostriatal DA transmission and PD rehabilitation using this model. In this research, we will test the following hypotheses centered on the endurance exercise, when administered at an early stage in the parkinsonian (PK) mice, will 1) improve their mobility and physical rehabilitation, 2) improve the efficacy of L-DOPA, 3) produce these effects by mechanistically causing an elevation of BDNF expression, an increase in the differentiation of DA progenitor cells, and an enhanced DA transmission and plasticity in the nigrostriatal neurons. Findings from this research should provide new insight into the development of alternative therapeutic approaches for enhancing the conventional pharmacological treatment and rehabilitation of PD. Potential benefits for using such a synergistic approach in managing PD would likely reduce the risk of drug toxicity and lower the cost of health

Principal Investigator: LI, JIA-YI
Grant Number: 5R21NS043717-02
Title: ADULT STEM CELL THERAPY IN PARKINSON'S DISEASE

Abstract: Objective: The aim of this project is to develop a novel source of adult stem cells as an alternative to embryonic-derived stem cells/tissue for neural grafting in Parkinson's disease (PD). Bone marrow-derived hematopoietic stem cells and brain-derived adult ependymal stem cells will be investigated with respect to their potential to differentiate into dopaminergic (DA-ergic) neurons in vitro: In a final phase, the cells will be grafted into PD animal models. The work program includes (phase 1) isolation and purification of both cell types by using specific markers and magnetic sorting or FACS. In phase 2, cells will be propagated in vitro and characterized; respective mitogenes screened and protocols optimized. Phase 3 involves the identification of factors promoting neuronal/DA-ergic differentiation for the respective cell types and optimization of differentiation protocols in vitro. Cells will be characterized morphologically by immunocytochemistry and functionally by measuring K+-stimulated DA release in vitro. Subsequently, to verify a possible clinical application of the investigated cell types for neural grafting, undifferentiated as well as differentiated cells will be transplanted intracerebrally in rat/mouse models of PD in phase 4. Grafted cells will be assessed morphologically by immunohistochemistry, their ability to form synaptic contacts with the host brain by staining for synaptic vesicle proteins (such as synaptophysin) in combination with confocal and electron microscopy. Functionality of grafted cells will be tested by rotational behavior pre- and post transplantation. The project is aimed at further understanding of neural stem cell biology and more importantly, to use a highly goal-derived approach to develop a translation protocol for adult-derived stem cells that can be readily applied in future clinical trials in PD. -

Principal Investigator: MUZYCZKA, NICHOLAS
Grant Number: 3P01NS036302-06A1S1
Title: Adeno-Associated Virus Gene Transfer to Nervous System

Abstract: Unavailable

Principal Investigator: MUZYCZKA, NICHOLAS
Grant Number: 2P01NS036302-06A1
Title: Adeno-Associated Virus Gene Transfer to Nervous System

Abstract: The long term goal of this Program is to develop gene transfer methods for the treatment of neural disorders. Three groups that are well integrated have come together to develop methods for using recombinant Adeno-associated virus (rAAV) for the treatment of retinal and CNS neurodegenerative diseases. Project 1 (Muzyczka) proposes genetic experiments to identify the proteins in the substantia nigra and striatum that interact with alpha synuclein. It will specifically examine alpha syn interactions with GRK and PLD2, and develop for the first time somatic knockouts of GRK and PLD2 using AAV vectors. It will also examine the effect of oxidative stress in combination with alpha syn overexpression on neurodegeneration in the substantia nigra. Finally, it will use biochemical techniques to directly identify protein complexes that contain alpha syn. Project 2 (Hauswirth and Lewin) will take the next step toward developing a therapy for P23H rhodopsin RP using the ribozymes they developed in the previous grant period. Further, they will test two new strategies for RP that are likely to be of more general use for all RP diseases. The first is the use of GDNF expression to promote photoreceptor survival. The second is to replace all (wild type and mutant) endogenous rhodopsin mRNAs with a wild type mRNA. If successful, this should prove to be a general approach that could be applied to all genetic RP, regardless of the point mutant involved. Project 4 (Mandel) will extend their preclinical experiments toward developing AAV mediated gene transfer for Parkinson disease. Specifically, they will develop regulatable GDNF constructs that are a prerequisite for clinical applications, do the first comprehensive analysis of the immune response to AAV vectors that are injected into the brain, and test their therapeutic GNDF strategy in a primate model of Parkinson's to obtain dosing information and confirmation of efficacy in a brain model closer to human. Two cores are also proposed. Core A (Administration) will continue in its role of providing fiscal/administrative support, educational programs, and program oversight in the form of internal and external advisors. The Vector Core will continue to improve the efficiency and scaleability of rAAV vectors. In addition to providing the routine service of production and purification of rAAV2-based vectors, the Core will also develop methods for purification of alternative AAV serotypes and capsid mutants to be used in projects 1, 2, and 3.-

Principal Investigator: Oldfield, Edward
Grant Number: 5Z01NS002813-15
Title: Drug Delivery Techniques

Abstract: Unavailable

Principal Investigator: PEREZ, RUTH G
Grant Number: 5R21NS045336-02
Title: Cell-based assays for neuroprotection in parkinsonism

Abstract: Unavailable

Principal Investigator: REDMOND, D EUGENE
Grant Number: 5P01NS044281-02
Title: Improving neural graft function in parkinsonian monkeys.

Abstract: The benefits of fetal neural transplantation in primate Parkinson's models have been partially confirmed by studies in patients, but transplantation may have significant problems which should be addressed. Functional improvement appears variable, less effective in older patients, and incomplete in spite of some apparent increases in dopamine production. The hypotheses are that transplantation's limitations result from inadequate grafts, due to poor survival of implanted cells, lack of critical growth factors, or nonphysiological graft placements and distribution. This program proposes to test these hypotheses with strategies which may improve functional benefits--the primary outcome measure of all studies in MPTP parkinsonian monkeys. Project One targets early cell death after grafting, with strategies to reduce oxidant stress, hypoxia/ischemia, and apoptosis using cell adhesion factors, the lazaroid tirilizad mesylate, melatonin, vascular endothelial growth factor, and cAMP. Project Two focuses on growth factors produced by fetal striatum enriched in astrocyte progenitor cells, or the growth factor, GDNF, delivered from encapsulated cells. An optimized method will be tested to determine benefits of combined methods in young adult and aged monkeys. Project Three aims to restore the relevant dopamine pathways by implantation of substantia nigra (SN) precursor tissue into SN and directing its outgrowth to the target areas, using co-grafted fetal striatal cells, or GDNF delivery. Duration of and stability of behavioral improvement, possible dyskinesias, or other toxic effects will be evaluated for three years and compared with striatal grafts. Quantitative behavioral effects will be correlated with biochemical and morphological measurements post-mortem. These studies may contribute to improving graft survival, reinnervation, and physiological restoration of the defective dopamine circuits and normalizing function. Although considerable preliminary work has been done in rodents, and because definitive controlled experiments with verifiable outcomes cannot be accomplished in humans, hypotheses and safety should be tested in the MPTP model in monkeys. The projects will be undertaken jointly by the program investigators, applying the resources of a unique primate transplantation laboratory (Core A) and shared outcome methodologies, all coordinated by a program support unit (Core B). Understanding of fetal precursor cell survival and outgrowth may also lead to improved understanding of the plasticity and function of other potential replacement cells, such as stem cells, and be relevant to other human neurodegenerative or traumatic conditions in addition to Parkinson's disease.-

Principal Investigator: REUBINOFF, BENJAMIN
Grant Number: 5R01NS046559-02
Title: Functional dopamine neurons from ES cells

Abstract: Parkinson's disease is a common neurodegenerative disorder that results from degeneration of dopamine (DA) neurons in the nigro-striatal system. Transplantation of fetal DA neurons can relieve Parkinsonism in some patients; however, limited tissue supply is a major obstacle for widespread use of fetal cells. Human embryonic stem (hES) cells could provide the platform for creating an unlimited supply of human DA neurons for cell therapy of Parkinson's disease. The goal of this study is to develop DA neurons from hES cells (NIH registration code ES01-06) and to demonstrate their function and therapeutic potential in animal models of Parkinson's disease. We have recently developed highly-enriched (>95 percent) cultures of expandable, developmentally competent neural progenitors (NPs) from hES cells. The NPs differentiate spontaneously into neurons expressing tyrosine hydroxylase (TH), however, at a low frequency. Our preliminary data suggest that defined signals can significantly promote the differentiation of hES cell-derived NPs towards TH+ neurons. In this study we will further develop the protocols to direct the differentiation of hES cells into TH+ neurons by the following approaches: (A) Administration of growth factors and cytokines that are known to induce a midbrain fate. (B) Forced expression of key transcription factors in the development of DA neurons. (C) Co-culture with stromal cells that have DA fate-inducing activity. Potential synergism between the strategies will be determined. We will evaluate whether hES cell-derived TH+ neurons have electrophysiological and functional properties expected from midbrain DA neurons and whether they can lead to recovery in the rat model of Parkinson's disease. Our preliminary results suggest that transplantation of hES cell-derived NPs to the DA-depleted striatum of rats results in behavioral recovery of DA-mediated motor asymmetry. Lastly, we will evaluate the potential of hES cell transplantation to correct behavioral deficits and the abnormal electrical activity of basal ganglia neurons in the MPTP primate model, which most reliably mimics the human disorder. This study will pave the way for further developments that may eventually allow the use of human ES cells as an unlimited source of midbrain neurons for transplantation in Parkinson's disease.-

Principal Investigator: SZETO, HAZEL H
Grant Number: 1R21NS048295-01
Title: Cell-Permeable Peptides for Mitochondrial Protection

Abstract: The application is submitted in response to the Program Announcement (PAR-02-138) requesting applications for exploratory/developmental projects in translational research. This proposal seeks to identify candidate therapeutics for neurodegenerative disorders. We have recently discovered a small lipophilic cationic peptide DAPL (Dmt-D-Arg-Phe-Lys-NH2, where Dmt = 2', 6'-dimethyltyrosine) that is cell permeable and selectively targets mitochondria. Preliminary studies with isolated mouse liver mitochondria have shown that this small peptide can protect against mitochondrial permeability transition and swelling, and reduce accumulation of reactive oxygen species. By protecting against mitochondrial dysfunction, this peptide may potentially be useful in the treatment of numerous neurodegenerative disorders. We are now seeking short-term support to further explore the pharmacology of this lead peptide analog in protecting brain mitochondria against various mitochondrial toxins, and to discover new analogs of this peptide that might lead directly to a therapy development project for a particular neurological disorder. Our specific aims are as follows: 1) To examine the ability of DAPL to protect mitochondria dysfunction caused by calcium overloading, 3-nitropropionic acid (3NPA), 1-methyl-4-phenylpyridium ion (MPP+), and t-butyl hydroperoxide (tBHP; 2) To examine the ability of DAPL to protect against cell death caused by glutamate, 3NPA, MPP +, and tBHP; 3) To carry out structure-activity relationship (SAR) studies with DAPL analogs to identify the optimal peptide analog for further preclinical development. The results from these exploratory studies will guide us to the development of preclinical animal studies for evaluating the therapeutic potential of DAPL analogs in the treatment of stroke and various neurodegenerative disorders, including Parkinson's disease, Huntington's disease and Alzheimer's disease. Potential collaborators for the animal studies have already been identified.-

Principal Investigator: TURNER, ROBERT S
Grant Number: 5R01NS044551-03
Title: DBS AND MOTOR CORTICAL FUNCTION IN AN MPTP MODEL OF PD

Abstract: Deep brain stimulation (DBS) of either the internal segment of the globus pallidus (GPO or the subthalamic nucleus (STN) is an effective treatment for most if not all symptoms of Parkinson's disease (PD). Several aspects of the reduction of symptoms with DBS provide tantalizing hints that different symptoms may be mediated by distinct pathways and/or physiological processes involving the motor and premotor cortices. The goals of this project are to use a non-human primate model of PD to gain a better understanding of the cortical mechanisms by which DBS produces clinical benefit, as well as to determine if different symptoms have different neuroanatomic/physiologic substrates. Animals will perform tasks that measure symptom-relevant behavioral parameters: movement selection/initiation/sequencing (akinesia), movement kinematics (bradykinesia), and rigidity. Neuronal activity at multiple locations in the four principal motor cortices [in different animals, primary motor (M1), ventral premotor (PMv), dorsal premotor (PMd), or mesial premotor (SMA)] will be monitored using a multielectrode array. Single cell activity will be assessed for changes in resting firing rate, task-related activity, and cell-to-cell interactions (synchronized firing) in response to DBS in GPI or STN before and after animals are rendered parkinsonian by intracarotid infusion of MPTP. The predictions are that: DBS-related changes in resting discharge will not be correlated with specific changes in symptoms. Increased activity and synchrony in SMA will be associated with reduced akinesia. Increases of the same in M1 will accompany reduced bradykinesia. Reductions in rigidity will be linked with a drop in M1 responses to passive movement and increased directional specificity in movement related activity. In addition, DBS may reduce abnormally-increased activity in PMv and PMd These hypotheses will be tested in three specific aims: Specific aim I will study the interacting effects of DBS and the type of motor task being performed. Specific aims 2 and 3 will identify cortical activities that change in concert with the time course (SA 2) and parametric relations (SA 3, DBS location, frequency, and strength) of symptom reduction with DBS. The results of these experiments will improve understanding of both the neuronal basis of different symptoms of PD and the mechanisms of action of DBS. Ultimately, these studies will advance a more complete pathophysiologic model of PD by incorporating the full array of parkinsonian symptoms.-

Principal Investigator: VITEK, JERROLD L
Grant Number: 7R01NS037019-06
Title: Deep Brain Stimulation in the Parkinsonian Monkey

Abstract: Over the last decade, the outlook for patients with advanced parkinsonism and other movement disorders has been revolutionized by the introduction of deep brain stimulation (DBS) in the subthalamic nucleus (STN) and internal segment of the globus pallidus (GPi) as a highly effective treatment modality. According to recent estimates over 2000 patients with PD have undergone implantation of DBS electrodes for the treatment of PD and over 15,000 patients per year may be candidates for this procedure. This number will increase, as the use of DBS as treatment of brain disorders becomes more widespread. Despite their widespread use, very little is known about the physiologic effects of DBS. Given the somewhat similar effect of lesions and stimulation in STN, GPI and thalamus on parkinsonian motor signs, it has been speculated that stimulation may act similar to lesioning, by blocking neuronal activity. Several studies have supported this view reporting suppression of neuronal activity in the site of stimulation. Our preliminary results, as well as the results of other groups have suggested that stimulation may, in fact increase output from the stimulated structure, demonstrating that stimulation in the STN increases neuronal activity in the GPi, while GPi stimulation suppresses neuronal activity in the thalamus. Additional support for this hypothesis is derived from microdialysis studies that found increased levels of glutamate in the entopeduncular nucleus (the rodent equivalent of GPi in primates) during STN stimulation. Conceivably, stimulation of basal ganglia activity may improve parkinsonism simply by regularizing pallidal discharge patterns. Both activation and inactivation could, in fact, be invoked during stimulation, because electrical stimulation may inhibit neuronal activity, while activating fibers in the stimulated area. For further optimization of current DBS protocols, and to minimize risks and side-effects of DBS implantation, it is mandatory that a solid understanding of the mechanism of action of this intervention is developed. This study will determine the mechanism underlying the effects of DBS of STN and GPi by examining in the MPTP monkey model of PD: 1) the effect of stimulation in the STN and GPi on neuronal activity and on neurotransmitter release in different portions of the basal ganglia-thalamocortical circuit, 2) the role of GPe in mediating the effect of stimulation in the STN and GPI, in mediating the development of parkinsonian motor signs and as an alternative site for stimulation for the treatment of PD and 3) determine the effect of stimulation in the STN and GPI on cortical function. The experiments will use a combination of single cell recording, microdialysis, and 18F-fluoro-deoxy-glucose

Principal Investigator: WETZEL, RONALD B
Grant Number: 5R01NS046356-02
Title: Conformational antibodies recognizing amyloid epitopes

Abstract: A number of neurological and other diseases are associated with the formation of protein aggregates called amyloid fibrils. Although the protein component of amyloid is different for different diseases, the three-dimensional shape of the amyloid seems to be the same in all cases. It has proven exceedingly difficult to get detailed structural information on amyloid, compromising our efforts to understand and devise treatments for Alzheimer's disease (AD), Parkinson's disease, Huntington's disease, and an array of other devastating conditions. We have developed antibody molecules that have the unique ability to bind to amyloid fibrils, regardless of the amino acid sequence of the constituent protein. Here we propose to develop these conformational antibodies as tools to improve our understanding of the amyloid structure and how it develops in human diseases. The specific aims are to (1) develop additional antibodies and antibody fragments, (2) structurally characterize these antibodies as a novel window onto the structure of amyloid fibrils, (3) characterize the fundamental basis of the antibody-amyloid interaction, and (4) develop the antibodies into tests for detecting amyloid in tissue and serum, as well as to monitor the emergence of the amyloid motif during in vitro amyloidogenesis. The tools involved in this work will include hybridoma and phage display technology, recombinant expression, mutagenesis, protein modeling and crystallography, immunochemical binding assays, and in vitro amyloid fibril assembly reactions. Since recently described vaccine approaches to amyloid diseases such as AD depend on the generation of antibodies, characterizing the structural basis of the anti-amyloid reaction is important. It is also important to understand the structure of amyloid and how these fibrils grow, as a means toward developing agents that will compromise fibril cytotoxicity and fibril growth. Finally, as a ubiquitous alternative folding pattern of protein polymers, amyloid and its structure is of fundamental importance to our understanding of the molecular basis of life. -

Principal Investigator: WOOTEN, MARIE W
Grant Number: 5R21NS044847-02
Title: Development of Par4 Peptide for Treatment of Alzheimers

Abstract: Unavailable

Principal Investigator: YUREK, DAVID M
Grant Number: 5R01NS042862-03
Title: Gene Therapy, Neural Grafts & Parkinson's Disease

Abstract: Clinical trials have provided encouraging evidence that grafts of fetal dopamine neurons are an effective therapeutic approach toward counteracting the symptoms of Parkinson's disease. Modest therapeutic benefits are observed in grafted patients despite clinical and experimental evidence that survival of grafted cells is low and graft reinnervation is incomplete. The poor survival and limited fiber outgrowth may be a consequence of neural grafts placed ectopically into an environment where the grafted neurons do not receive the proper signals for successful growth and integration into the neural circuitry of the host brain. Gene therapy may be a viable technique to introduce factors [neurotrophic factors] into brain tissue that can potentiate the survival and functional outgrowth of neural grafts, and thus improve the therapeutic value of the graft. In the proposed studies, regulated viral vectors will be injected into the lesioned nigrostriatal pathway of rodents with experimental Parkinson's disease in order to induce transgene expression of several neurotrophic factors that have a history of providing potent neurotrophic support for dopamine neurons. Subsequently, neural grafts will be implanted into lesioned/transduced brain sites and the survival, reinnervation, and function of the grafts will be assessed. Because Parkinson's disease has a higher incidence in the elderly than in the younger population, and recent experimental evidence suggests that the expression of endogenous neurotrophic factors are diminished in the aged striatum following a neurodegenerative lesion, experiments will be performed in young, middle-age, or old rats with experimental Parkinson's disease and the results will be compared within and between each age group. The studies are designed to determine the optimal temporal expression of neurotrophic factors [GDNF, BDNF, FGF-2] that improve graft development and function using regulated viral neurotrophic factors [GDNF, BDNF, FGF-2] that improve graft development and function using regulated viral vectors in young and aged animals with experimental Parkinsonism. These studies will also determine if combinations of viral vectors expressing different neurotrophic factors can be used to improve the therapeutic effects of dopamine grafts.-

Principal Investigator: ZAMORE, PHILLIP D
Grant Number: 5R21NS044952-02
Title: RNAi as a Potential Therapy for ALS

Abstract: Unavailable

Principal Investigator: ZHANG, SU-CHUN
Grant Number: 5U01NS046587-02
Title: Stem Cell Therapy For Parkinson's Disease

Abstract: Parkinson's disease (PD) results from the progressive loss of dopamine (DA) neurons in the midbrain. Replacement of the lost DA neurons with fetal midbrain cells through neural transplantation in clinical trials has produced clinical benefits and has laid a foundation for cell therapy in PD. This therapy, however, is hindered by the limited supply of effective donor cells. Human embryonic stem (hES) cells (NIH Registry WA01 and WA09), established from the inner cell mass of a preimplantation embryo, are capable of almost unlimited proliferation in an undifferentiated state, yet retain the potential to differentiate into almost all cell and tissue types of the body including DA neurons. Thus ES cells may provide a simple and continual source of specialized human cells, which can be standardized and banked. This application is to resolve a single but crucial issue surrounding potential stem cell replacement therapy for PD, i.e., which hES-derived cell type, neuroepithelial cells, DA neuron progenitors, or DA neurons, is best for transplant therapy in PD. This study is based on our success in guiding hES cells to neuroepithelial cells, DA neuron progenitors and mature DA neurons in culture. The criteria for determining the candidate cell type include safety to recipients, efficacy of the cells for functional replacement, efficiency in cell production, and simplicity for standardization of cell preparation procedures. The proposed study will determine the ideal hES-derived cells for PD therapy, thus leading to preclinical studies to transplant the selected cells into a monkey PD model we have established, and to bank and/or standardize the cell production in our Biomanufacturing Facility before clinical trials.-

Principal Investigator: ZHOU, JIANHUA
Grant Number: 5R21NS045351-02
Title: A cell base system for compounds regulating tau splicing

Abstract: Unavailable

Principal Investigator: Ziemba, Kristine S
Grant Number: 1F30NS048716-01
Title: Reconstruction of the nigrostriatal pathway

Abstract: The long-term objective of this research proposal is to develop a means to reconstruct the neural circuit that degenerates in Parkinson's disease (PD) - that is, the nigrostriatal pathway. While current therapy (levadopa treatment) for PD may alleviate symptoms for a while, there is still no way to halt or reverse the neurodegeneration. Since 1% of the population over the age of 65 is affected by PD, and the prevalence increases with increasing age, research into better therapies and an eventual cure for PD is important for our aging population. Cellular replacement is not a new idea in PD research, but this proposal differs from most previous efforts by attempting an anatomically and physiologically correct reestablishment of the nigrostriatal pathway, effecting a more complete behavioral recovery. Molecular cues to guide growth of dopaminergic neurons will be identified in vitro, and adenoviral vectors will be used to express these molecules between the substantia nigra (SN) and the striatum in hemiparkinsonian rats. When dopaminergic neurons are subsequently transplanted into the SN, their axons should grow along the growth-supportive pathway, ending in the striatal target. Success will be evaluated with detailed histological and behavioral analyses.-